

Docket No. 875.030US1



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METHOD OF RAPIDLY GENERATING DOUBLE-STRANDED RNA AND METHODS OF
USE THEREOF

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To generate p2rRNAProm (Fig. 1A), a 292-bp fragment containing the *T. brucei* rRNA promoter was PCR-amplified with primers that added *Xba*I and *Bam*HI sites to the ends and inserted into the *Sal*II and *Bam*HI sites of pHD496 in the opposite orientation to the rRNA promoter already present (Biebinger S, et al. (1996) *Nucleic Acids Res* 24:1202-11). Plasmid p2rRNAProm/αtub was created by inserting a 486-bp PCR fragment of *T. brucei* α-tub (60 bp of the 5' UTR and 426 bp of coding region) into the *Hind*III and *Bam*HI sites of p2rRNAProm. A second T7 promoter in the opposite orientation to the T7 promoter already present was added to pBluescriptII SK(-) by annealing oligos 5'-CGTAATACGACTCACTATAGGGCAGCT-3' (SEQ ID NO:1) and 5'-GCCCTATAGTGAGTCGTATTACGAGCT-3' (SEQ ID NO:2) and ligating into the *Sac*I site of pBluescriptII SK(-) to give p2T7 (Fig. 1A).

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